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# Cooperation of oncolytic virotherapy with VEGF-neutralizing antibody treatment in IDH wildtype glioblastoma dependends on MMP9

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## Letter to the editor

Development of efficacious immunotherapy approaches in glioblastoma remains challenging. The induction of anti-glioma immune responses utilizing various oncolytic virus strains has been reported from pre-clinical and early phase clinical trials.[1] The optimal design for efficacy studies of oncolytic virotherapy in glioblastoma patients is under debate, but likely such studies will preferentially be performed in patients with recurrent glioblastoma.

In clinical practice, this group of patients is often treated with the anti-angiogenic vascular endothelial growth factor A (VEGF)-neutralizing antibody bevacizumab for its capacity to improve and maintain quality of life, mainly through anti-edematous effects. Beyond promoting angiogenesis, VEGF exerts immunosuppressive effects,[2] which are thought to be potentially relevant in the context of immunotherapy in glioblastoma. For example, in the Re-ACT trial, median overall survival of patients with recurrent glioblastoma was prolonged by 3.2 months upon addition of a tumor-specific vaccine to bevacizumab (hazard ratio = 0.47, p=0.021) and benefit was associated with vaccine-induced titers within the experimental arm,[3] but in the ACT-IV trial the same vaccine had no effect on outcome as an add-on to standard chemoirradiation in patients with newly diagnosed glioblastoma irrespective of titer generation in response to vaccination.[4]

This led us to explore whether bevacizumab should be incorporated into virotherapy clinical trial designs in glioblastoma. We employed a non-immunogenic genetic model of IDH wildtype glioblastoma which is histologically, immunologically and molecularly highly similar to its human counterpart (*N/tv-a;Cdkn2a*$^{-/-}$

*;Pten$^{fl/fl}$:PDGF,Cre*).[5] Animal research was prospectively approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center, Seattle, WA (Animal assurance institutional #A3226-01; Protocol #50842). Mouse glioblastomas were treated with oHSV$^{ULBP3}$, an oncolytic herpes simplex virus 1 strain that was armed with a *ULBP3* gene expression cassette to boost anti-cancer immunity (Figure 1A).[5] There was a twofold increase in VEGF protein levels upon treatment with oHSV$^{ULBP3}$ versus PBS in protein lysates from 5 mouse glioblastomas per group (Figure 1B). A major proportion of VEGF is, however, immobilized in the extracellular matrix. In order to exert its effects, immobilized VEGF isoforms need to be cleaved by matrix metalloproteinases (MMP), predominantly MMP3 and MMP9.[6] Although there was an about threefold increase in MMP9 protein levels upon treatment with oHSV$^{ULBP3}$, our model reflects a scenario of overall low MMP expression (Figure 1B). In order to model effects of MMP expression on the efficacy of oHSV$^{ULBP3}$, we designed an oHSV vector containing an *MMP9* expression cassette in addition to the *ULBP3* cassette, yielding *MMP9* expression specifically in the small virus-infected foci of the tumor. The survival benefit of tumor-bearing mice obtained with oHSV$^{ULBP3}$ versus PBS (HR 0.27, $p<0.001$) was nearly abolished in mice randomized to intratumoral injection with oHSV$^{ULBP3\text{-}MMP9}$ (HR=0.56, p=0.039, Figure 1C). Co-treatment of mice with the murine VEGF neutralizing antibody B20 and intracranial injections of PBS, oHSV$^{ULBP3}$ or oHSV$^{ULBP3\text{-}MMP9}$ indicated that VEGF was a major driver of reduced therapeutic efficacy of oHSV$^{ULBP3}$ in the presence of MMP9. Consistent with clinical trials of anti-VEGF treatment in glioblastoma patients, there was no effect of B20 on mouse survival (HR 0.86, p=0.71), but combining oHSV$^{ULBP3\text{-}MMP9}$ with B20 resulted in a marked survival benefit compared to oHSV$^{ULBP3\text{-}MMP9}$ alone (HR = 0.32, log rank

p=0.027, Figure 1D). By contrast, no effect of B20 was observed upon treatment with oHSV$^{ULBP3}$ lacking the MMP-9 expression cassette (HR 1.11, p=0.83, data not shown). In line with the notion of an immunosuppressive effect of MMP9 and subsequent VEGF release, nCounter geneset analysis revealed down-regulation of the genesets toll-like receptor (TLR) signaling (p<0.001) and T-cell activation and checkpoint signaling (p=0.001) in tumors treated with oHSV$^{ULBP3\text{-}MMP9}$ versus oHSV$^{ULBP3}$ (Figure 1E,F), indicating inhibitory effects on both the myeloid and lymphocytic compartment. Interestingly, disruption of autocrine VEGF signaling in tumor cells results in loss of MMP9 expression, consistent with a positive feedback loop between VEGF and MMP9.[7]

In summary our data suggest a mechanism of resistance to virotherapy that involves VEGF release from the extracellular matrix by MMP9 and support a clinical trial design that incorporates the combination of virotherapy and bevacizumab. The unfavorable effect of MMP9 on the efficacy of virotherapy argues against oncolytic vector designs that incorporate MMP9 cassettes to improve viral spreading.

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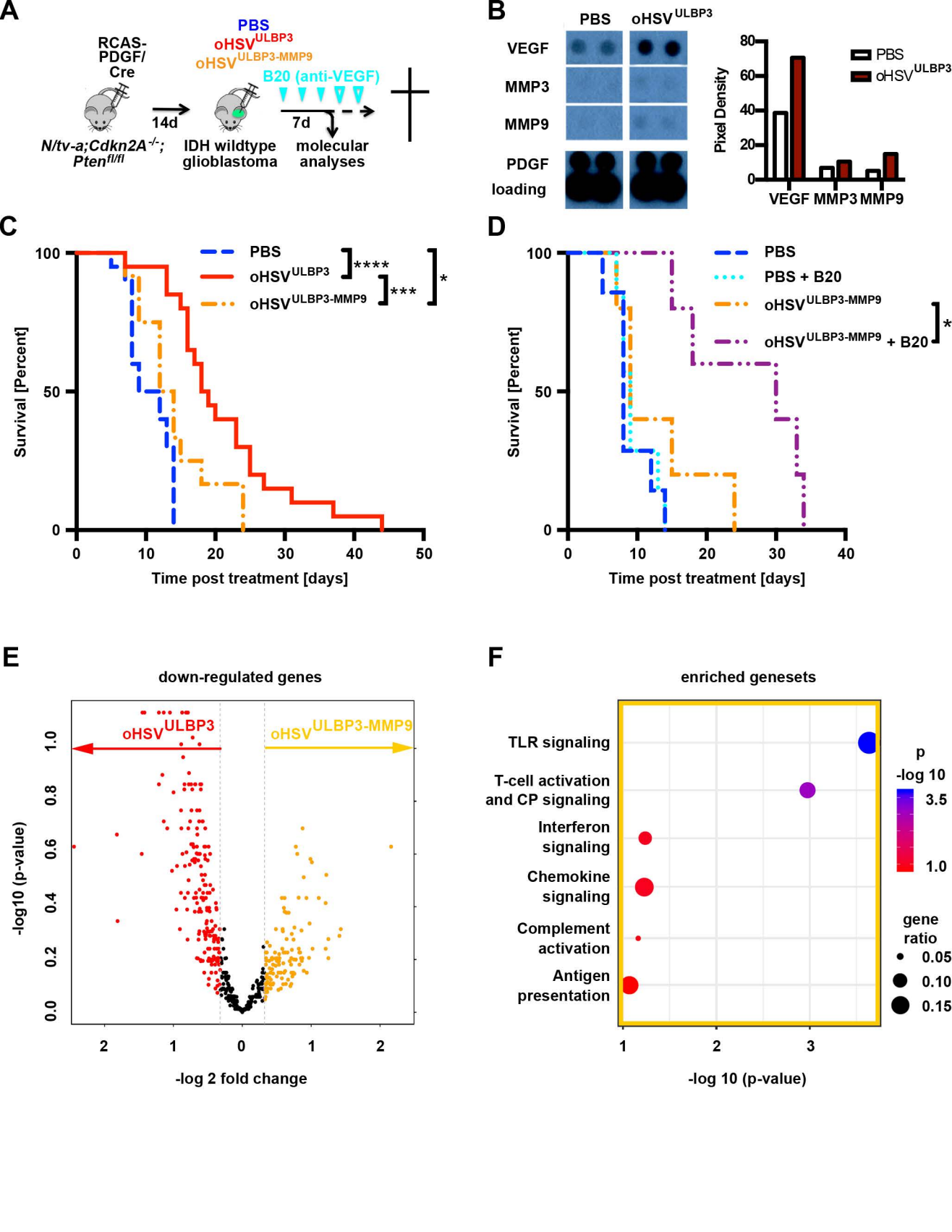
**Figure legend**

Figure 1. Anti-VEGF counteracts MMP9-mediated resistance to virotherapy.

A. Experimental setup. B. Proteomics analysis (R&D ARY028) of pooled tumor lysates (N=5 per group) from *N/tv-a;Cdkn2a*$^{-/-}$*;Pten*$^{fl/fl}$*:PDGF,Cre* glioblastomas treated by intracranial injection of PBS or oHSV$^{ULBP3}$ as indicated. C. Symptom-free survival. PBS, N=20; oHSV$^{ULBP3}$, N=20; oHSV$^{ULBP3\text{-}MMP9}$, N=12. Kaplan-Meier curves were compared utilizing the log rank test. D. Symptom free survival. PBS alone N=7, co-treatment with B20 (anti-VEGF; N=7, 5 mg/kg i.v. every other day), or upon intratumoral injection with oHSV$^{ULBP3\text{-}MMP9}$ ($1x10^6$ PFU) alone (N=5) or in combination with B20 (N=5). Kaplan-Meier curves were compared utilizing the log rank test. E. Volcano plot of differentially down-regulated genes analyzed by nCounter analysis of the myeloid innate immunity geneset after treatment with oHSV$^{ULBP3}$ or oHSV$^{ULBP3\text{-}MMP9}$. F. Gene set enrichment analysis of genes down-regulated upon injection with oHSV$^{ULBP3\text{-}MMP9}$ versus oHSV$^{ULBP3}$ (orange in panel E).


A
RCAS-PDGF/ Cre
PBS
oHSV ULBP3
oHSV ULBP3-MMP9
B20 (anti-VEGF)
14d
7d
N/tv-a;Cdkn2A -/-; Pten fl/fl
IDH wildtype glioblastoma
molecular analyses
B
PBS
oHSV ULBP3
VEGF
MMP3
MMP9
PDGF loading
Pixel Density
PBS
oHSV ULBP3
VEGF MMP3 MMP9
C
PBS
oHSV ULBP3
oHSV ULBP3-MMP9
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*
Survival [Percent]
Time post treatment [days]
D
PBS
PBS + B20
oHSV ULBP3-MMP9
oHSV ULBP3-MMP9 + B20
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Survival [Percent]
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E
down-regulated genes
oHSV ULBP3
oHSV ULBP3-MMP9
-log10 (p-value)
-log 2 fold change
F
enriched genesets
TLR signaling
T-cell activation and CP signaling
Interferon signaling
Chemokine signaling
Complement activation
Antigen presentation
p -log 10
gene ratio
-log 10 (p-value)